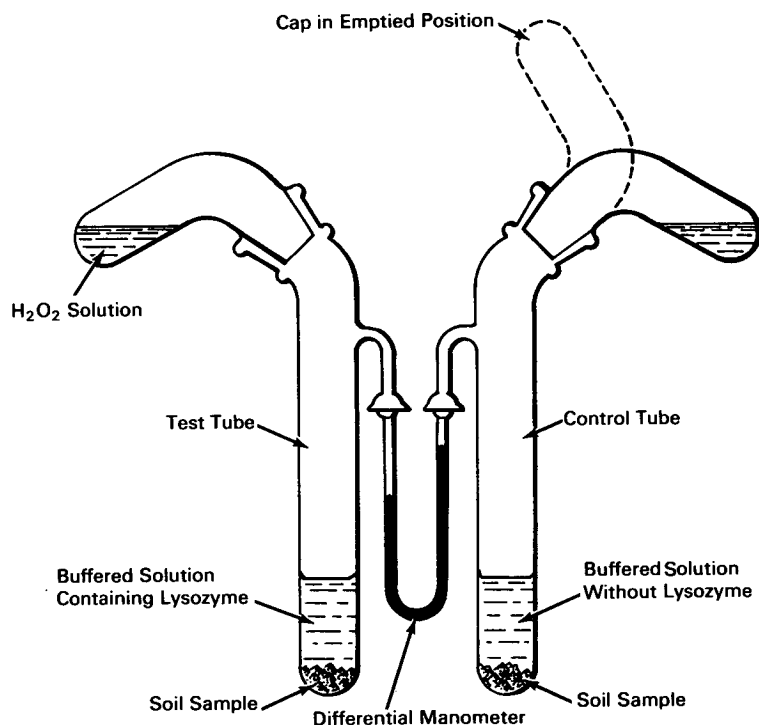


NASA TECH BRIEF



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Microorganisms Detected by Enzyme-Catalyzed Reaction



The problem:

To devise a simple, rapid method for detecting the presence of microorganisms in soils. The method must not require preliminary separation of the microorganisms from soil samples or extraction and analysis of soluble products from a reaction mass.

The solution:

The enzyme lysozyme (muramidase) is used to release the enzyme catalase from the microorganisms (most types of bacteria) present in the soil sample. The

catalase catalyzes the decomposition of added hydrogen peroxide to produce oxygen which is detected manometrically. The partial pressure of the oxygen serves as an index of the sample's bacterial content.

How it's done:

The soil to be tested is ground to a homogeneous consistency with a mortar and pestle. One-gram samples of the homogeneous soil are placed in each of the two reaction tubes, one of which serves as a control.

(continued overleaf)

A measured volume of buffered lysozyme solution is added to the test tube and an equal volume of the buffer solution (without lysozyme) is added to the control tube. After allowing 20 minutes for the lysozyme to lyse (disintegrate) the bacterial cells present in the soil, equal volumes of hydrogen peroxide previously placed in the caps on each of the reaction tubes are drained into the tube contents. If any bacteria are present in the soil contained in the reaction tubes, the pressure of the oxygen produced by the catalase reaction will be indicated by the difference in level of the liquid surfaces in the U-tube manometer.

Notes:

1. Initial experiments to demonstrate the principle of the method were carried out in the simple apparatus illustrated above. More precise determinations were carried out at 22°C in a Warburg shaker apparatus. Single side-arm Warburg reaction flasks were attached to both legs of a Warburg manometer so that differential pressure measurements could be made.
2. A more precise differential manometer can be used to increase the measurement sensitivity. Smaller quantities of released oxygen can be detected by pressure transducers, fluorescence quenching, and the luciferase-luciferin reaction.

3. The method may also be used for detecting the presence of bacteria in water, dust, fabrics, and other materials. Other enzymes or mechanical devices may be used for disintegrating more varieties of bacteria.

4. Further information concerning this method is given in *JPL Space Program Summary No. 37-38*, vol. IV, 31 August 1964, p. 115-118; and "*Detection of Micro-Organisms in Soil by Their Catalytic Activity*", *Nature*, vol. 106, p. 1019-1021, 5 June 1965.

Inquiries may also be directed to:

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Reference: B66-10117

Patent status:

Inquiries about obtaining rights for the commercial use of this invention may be made to NASA, Code GP, Washington, D.C., 20546.

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